

## EFFECT OF HYPOXIA ON LEARNED AND MOTOR RESPONSES:

## Pharmacotherapeutic Approaches to Hypoxia

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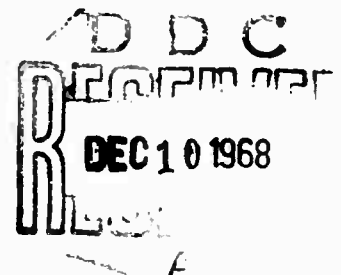
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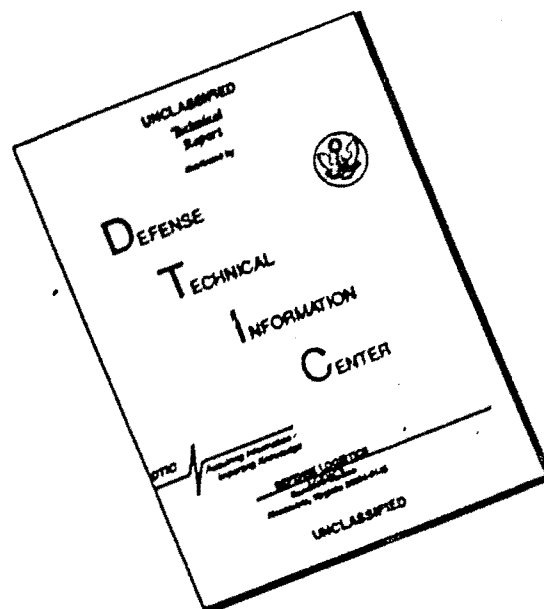
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## FOREWORD

During the past year, we have continued to investigate the psychological and physiological factors which determine performance in reduced oxygen atmospheres. We have carried on several types of investigations which can be categorized as follows:

(1) The effects of carbon dioxide and/or carbonic anhydrase inhibition on performance and neural reactivity in low oxygen environments.

(2) The effects of carbon dioxide and/or carbonic anhydrase inhibition on blood gases and acid-base balance in low oxygen environments.

(3) The effects of "motivation" on performance in low oxygen environments.

(4) The effects of past experience in low oxygen upon performance in subsequent exposures.

(5) The effects of schedules of reinforcement on performance in low oxygen.

(6) The "conditionability" of the deleterious effects of hypoxia, i.e. the association of previously "neutral" environmental stimuli with the deleterious effects of hypoxia. ( ) ←

The results of each category of investigation have been treated in published reports or are in preparation. These reports and manuscripts are appended to this document.

In this annual report, we will describe the most significant aspects of our work with detailed documentation included in the appendices.

I

# I. THE EFFECTS OF CARBON DIOXIDE AND/OR CARBONIC ANHYDRASE INHIBITION ON PERFORMANCE AND NEURAL REACTIVITY IN LOW OXYGEN ENVIRONMENTS.

## A. The Effects of Carbon Dioxide on Hypothalamic Self-stimulation During Hypoxia

Miescher-Rusch in 1885 attributed an angelic role to carbon dioxide when he stated "over the oxygen supply of the body, carbon dioxide spreads its protecting wings." Since that time, a number of investigators have either suggested or demonstrated that carbon dioxide does indeed partially protect the body from the effects of hypoxia. Winterstein (1934) suggested that carbon dioxide inhalation might be helpful in maintaining respiration and promoting oxygen absorption at high altitude. In 1935, Child, et al. concluded that:

"At least up to 4,000 feet, B.P. 450 mm Hg inhalation of a small amount of carbon dioxide within the inspired air is distinctly beneficial in protecting against both acapnia and anoxia during and after vigorous physical exertion. As the effects were even better on those who had made no great exertion, we suggest that such an inhalation might be of considerable value also for passengers traveling by air up to at least 14,000 feet."

Gellhorn in 1935 demonstrated deleterious effects of both carbon dioxide lack and excess upon audition. In 1937, he reported the beneficial effects of the addition of carbon dioxide during hypoxia. He attributed this improved performance to the circulatory and thermoregulatory effects of carbon dioxide inhalation. In 1943, Gibbs, et al. once again emphasized the importance of carbon dioxide in maintaining normal cortical function and in an elegant set of experiments demonstrated the effects of the addition of five percent carbon dioxide on problem solving in humans breathing ten percent, eight percent, six percent and four percent O<sub>2</sub>. This data was not reported in any detail. He simultaneously collected arterial and jugular venous blood samples and recorded EEG. A very recent report by Gerben (1968) demonstrates an increase in activity

level upon inhalation of five percent carbon dioxide by hypoxic rats and a depression of activity level upon inhalation of five percent carbon dioxide by normoxic animals.

We recently reported (1967) that the rate of hypothalamic self-stimulation was a function of the inspired oxygen concentration. When current levels were sufficient to produce "high" rates of self-stimulation (100 resp./min.), there was a graded relationship between inspired oxygen and response rate. This relationship was markedly affected by alterations in stimulus intensity. If current levels were reduced sufficiently to produce response rates of 50 per minute, mild and severe hypoxia produced virtually equal reductions in self-stimulation rates.

A theoretical interpretation of this phenomenon was presented based on the postulated existence of a sigmoid function relating stimulus intensity and self-stimulation rate (Olds, 1961). The increased self-stimulation produced by increased current levels is mediated through the activation of a larger proportion of the potentially effective neuronal pool. The flattening of the curve occurs when the pool is maximally active, and increased current brings in non-effective, sometimes interfering neuron populations.

Hypoxia produces a series of changes in the activity of neural tissues which have been extensively described by Creutzfeld and his collaborators. The transient activation of single neurons in Creutzfeld's second or "activation" period is gradually replaced by the inactivation of an increasing percentage of the effective neuronal pool. This inactivation, associated with a depolarization of the membrane potential and a blocking of synaptic transmission is probably responsible for the decrement in self-stimulation rates. Creutzfeld states that there were no major differences

between the neural effects of anoxia produced by circulatory arrest and that the accumulation of carbon dioxide in the instance of circulatory arrest has no marked effect on the measured parameters of neural function.

We have investigated the effects of carbon dioxide on self-stimulation in hypoxia in order to establish in a quantitative manner the physiological role that carbon dioxide plays in the maintenance of a functional neuronal population. This is discussed in light of the circulatory, respiratory and acid-base effects of carbon dioxide alterations. (See Appendix A)

Figures 1 - 4 illustrate the mean rate of responding for the eight animals for two-minute periods, under the experimental and control conditions. As can be seen From Figure 1, when no carbon dioxide is administered, there is a direct relationship between rate of lever pressing and level of inspired oxygen. Animals in 12 percent oxygen lever press significantly slower ( $p < .05$ ) than animals in 14 percent. The 10 percent oxygen animals are significantly slower ( $p < .001$ ) than the 12 percent animals, and the 8 percent animals are slower ( $p < .005$ ) than the 10 percent animals. These results confirm data reported in our previous paper (Annau and Weinstein, 1967) dealing with the relationship between self-stimulation rates and hypoxia. (See Appendix B)

The addition of one percent  $\text{CO}_2$ , illustrated in Figure 2, improves the performance of all groups during hypoxia. The 12 percent  $\text{O}_2$  group improves the level of the 14 percent  $\text{O}_2$  group. The 10 percent oxygen group is still significantly slower ( $p < .001$ ) than the 12 percent group, and the 8 percent group is slower than the 10 percent  $\text{O}_2$  group ( $p < .05$ ). Figure 3 illustrates the performance of the four groups with the addition of two percent carbon dioxide. The increased  $\text{CO}_2$  of the inspired gas raised the level of performance of the 10 percent oxygen group to the

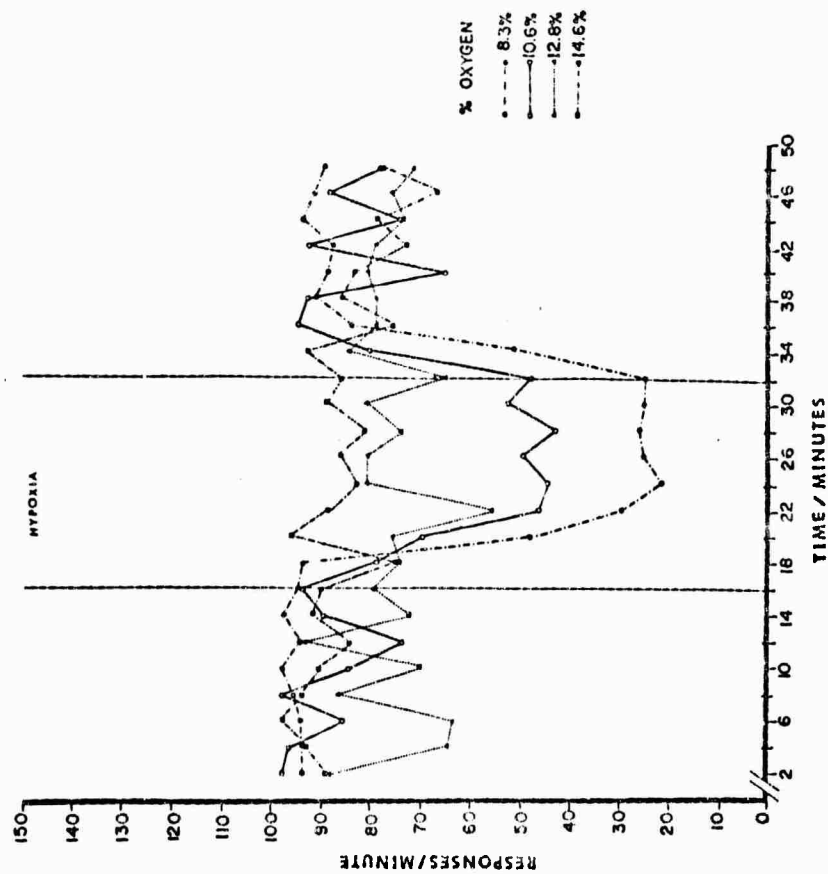


FIGURE 1. LEVER PRESSING RATES FOR SUCCESSIVE TWO-MINUTE PERIODS IN AIR 10-16 MIN) IN HYPOXIA (16-32 MIN) AND IN AIR 132-48 MIN) WITH NO CO<sub>2</sub> PRESENT DURING THE HYPOXIC PERIOD. EACH CURVE REPRESENTS THE MEAN PERFORMANCE OF THE SAME SIX ANIMALS RUN EACH DAY IN A CONTROL PERIOD IN AIR FOLLOWED BY A SIXTEEN-MINUTE PERIOD IN HYPOXIA AND A SIXTEEN-MINUTE RECOVERY PERIOD IN AIR.

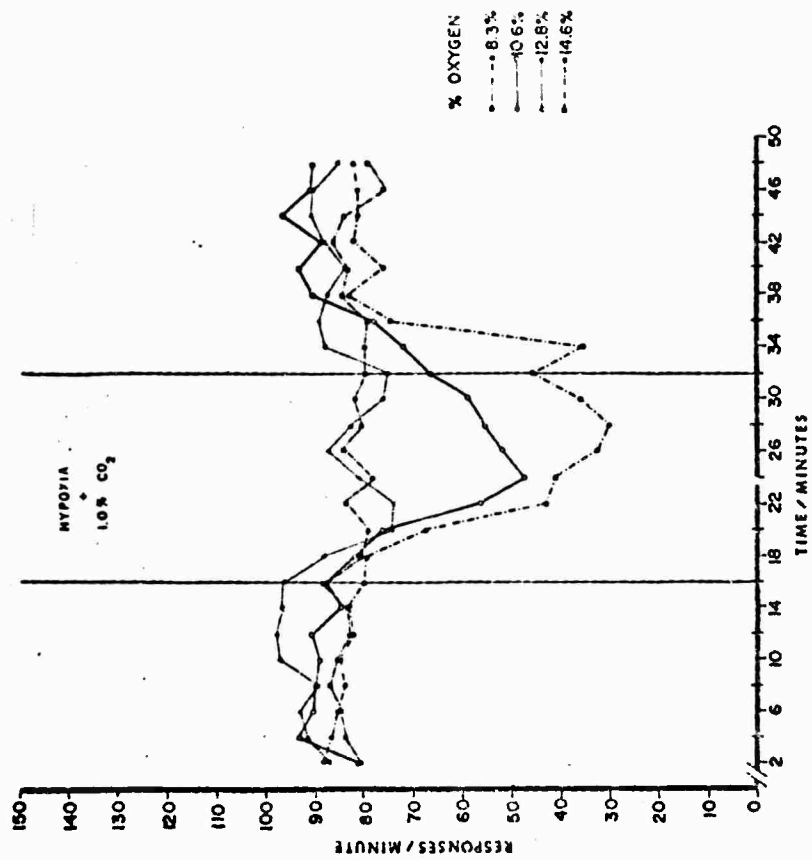


FIGURE 2. THE EFFECT OF 1 PERCENT CO<sub>2</sub> ON SELF-STIMULATION RATES DURING HYPOXIA. SUCCESSIVE LEVER PRESSING RATES ARE SHOWN IN AIR 10-16 MIN) IN HYPOXIA AND 1 PERCENT CO<sub>2</sub> (16-32 MIN) AND IN AIR 132-48 MIN).

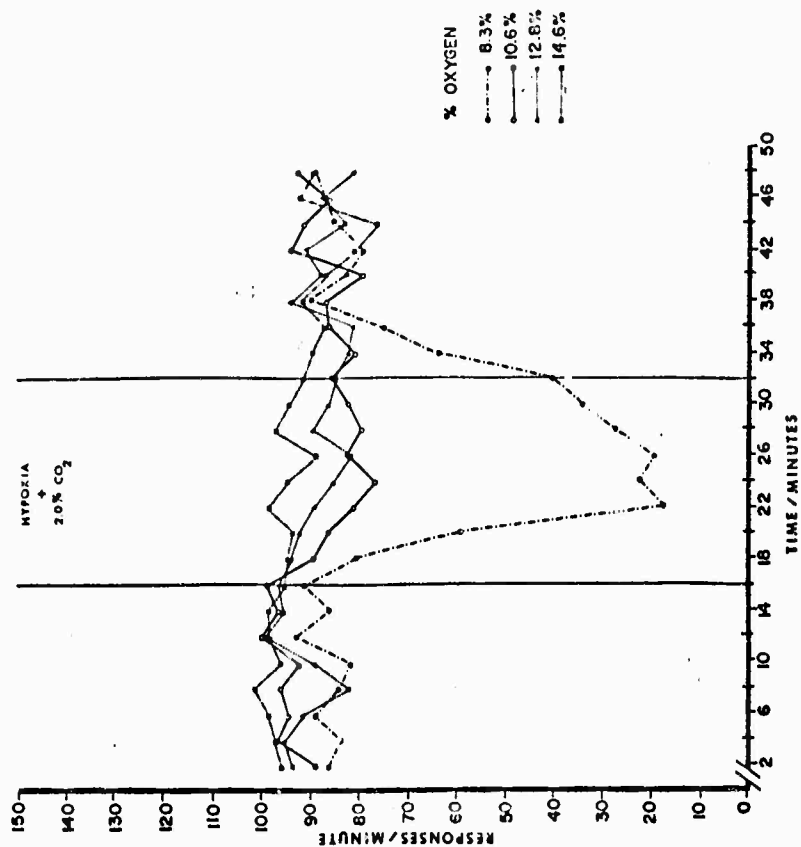


FIGURE 3. THE EFFECT OF 2 PERCENT CO<sub>2</sub> ON SELF-STIMULATION RATES DURING HYPOXIA. SUCCESSIVE LEVER PRESSING RATES ARE SHOWN IN AIR (0-16 MIN) IN HYPOXIA AND 2 PERCENT CO<sub>2</sub> (16-32 MIN) AND IN AIR (32-48 MIN).

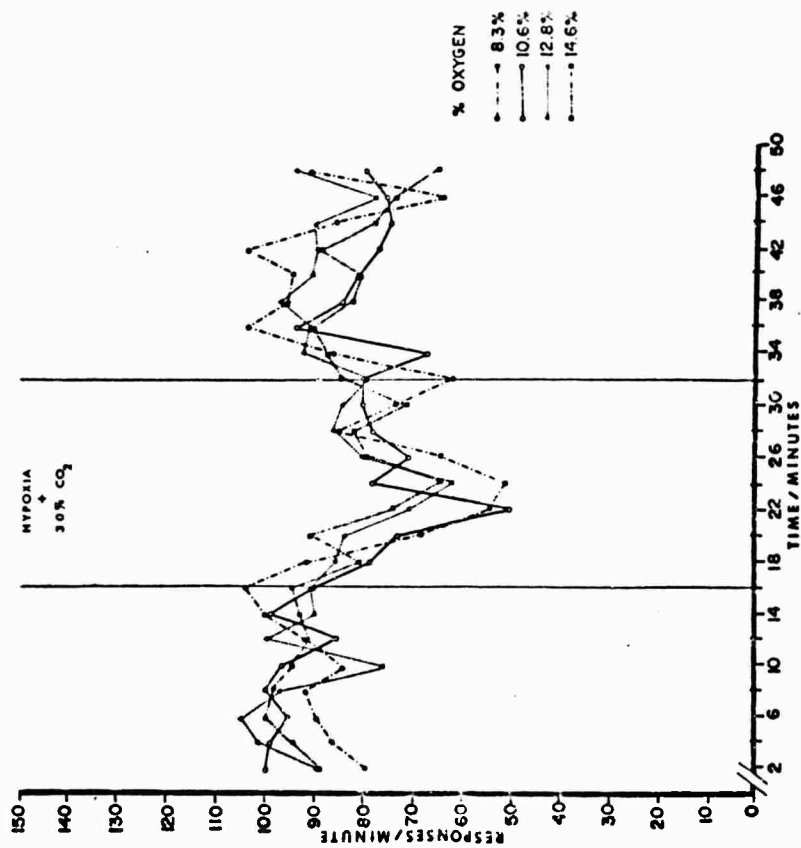


FIGURE 4. THE EFFECT OF 3 PERCENT CO<sub>2</sub> ON SELF-STIMULATION RATES DURING HYPOXIA. SUCCESSIVE LEVER PRESSING RATES ARE SHOWN IN AIR (0-16 MIN) IN AND 3 PERCENT CO<sub>2</sub> (16-32 MIN) AND IN AIR (32-48 MIN).

level of the 12 percent group. The 14 percent oxygen group also improved significantly ( $p < .008$ ) above the level of the 12 percent oxygen group. The 8 percent oxygen group is significantly slower than all groups ( $p < .001$ ). The addition of 3 percent  $\text{CO}_2$ , illustrated in Figure 4, brings the level of performance of all groups together. No significant differences in performance remain between the groups. There is, however, a slight depression in the lever pressing rates of the animals in 14 percent and 12 percent  $\text{O}_2$  when compared with their performance in 2 percent  $\text{CO}_2$ . The animals breathing 14 percent oxygen and 3 percent carbon dioxide have significantly lower rates ( $p < .03$ ) than in 14 percent oxygen and 2 percent  $\text{CO}_2$ .

The results indicate that each 1 percent addition of carbon dioxide improves the performance of the hypoxic animals towards control rates in 21 percent oxygen. A series of paired comparisons between the carbon dioxide and non-carbon dioxide conditions indicate that with the addition of 2 percent carbon dioxide, animals breathing 8 percent oxygen perform at the same level as animals breathing 10 percent oxygen with no carbon dioxide. With 3 percent carbon dioxide, the performance level of the 8 percent oxygen animals reaches the performance level of animals breathing 12 percent oxygen without carbon dioxide. Similarly, the addition of 1 percent carbon dioxide improves the animals in 10 percent oxygen to the level of the 12 percent group, and with 2 percent carbon dioxide, they perform as well as animals breathing 14 percent oxygen without carbon dioxide. Finally, addition of 1 percent carbon dioxide improves the performance of the 12 percent oxygen group to the level of the 14 percent oxygen group. These results indicate that at the higher oxygen concentrations (10 % and 12%), the addition of 1 percent carbon dioxide causes an improvement in performance equivalent to an increment of 2 percent inspired oxygen. When the level of inspired oxygen is very low, as in 8 percent

oxygen, a 2 percent increase in carbon dioxide concentration is required to produce a behavioral change equivalent to a 2 percent increase in oxygen.

Our data demonstrate clearly that the addition of carbon dioxide to hypoxic gas mixtures produces significant protection against the deleterious effects of diminished inspired oxygen. The amount of protection is a function of the carbon dioxide concentration, with greater protection being afforded by increasing carbon dioxide levels.

There are several possible mechanisms for the beneficial action of carbon dioxide on neural function in hypoxia. Carbon dioxide is a potent ventilatory stimulus and as such produces hyperventilation which serves to bring the alveolar oxygen tension closer to the inspired oxygen. This rise in alveolar oxygen produces an increase in arterial, and subsequently in tissue-oxygen partial pressure. Carbon dioxide is also a potent cerebrovasodilator producing an increased circulation to neural tissue. Under hypoxic conditions, the increased circulation provides a major improvement in brain tissue oxygen partial pressure. A third effect of the carbon dioxide is to produce an increased hydrogen ion concentration, thus preventing the respiratory alkalosis which is the normal concomitant of hypoxia. This effect acts to increase both ventilation and cerebral blood flow.

The relationship between stimulus intensity and self-stimulation rate is best described by a sigmoid function. This function describes the sequential activation of neurons in a population of cells mediating motivational effects. These cells are distributed around capillaries in a manner which produces an effective oxygen gradient among them, i.e. some cells are close to a capillary and have a high  $pO_2$ , whereas some are far from the capillary and have a low  $pO_2$ . Likewise, some are near

the arterial end while some are near the venous end, producing another source of oxygen gradient. Neurons furthest from capillaries and at the venous end are most hypoxic and will show the greatest impairment in functions, which can consist of an increase in conduction time and synaptic delay, and a decreased threshold followed by complete depolarization.

During hypoxic exposures, an increasing number of neurons begin to show impairment with their individual susceptibilities being determined by their position relative to capillaries and metabolic rate. Neurons farthest from the points of high current density may be fired during air breathing, but when hypoxic become incapable of generating action potentials. It is clear that the size of the functional neuronal pool mediating motivated behavior will be a function of stimulus intensity, oxygen distribution and their interaction.

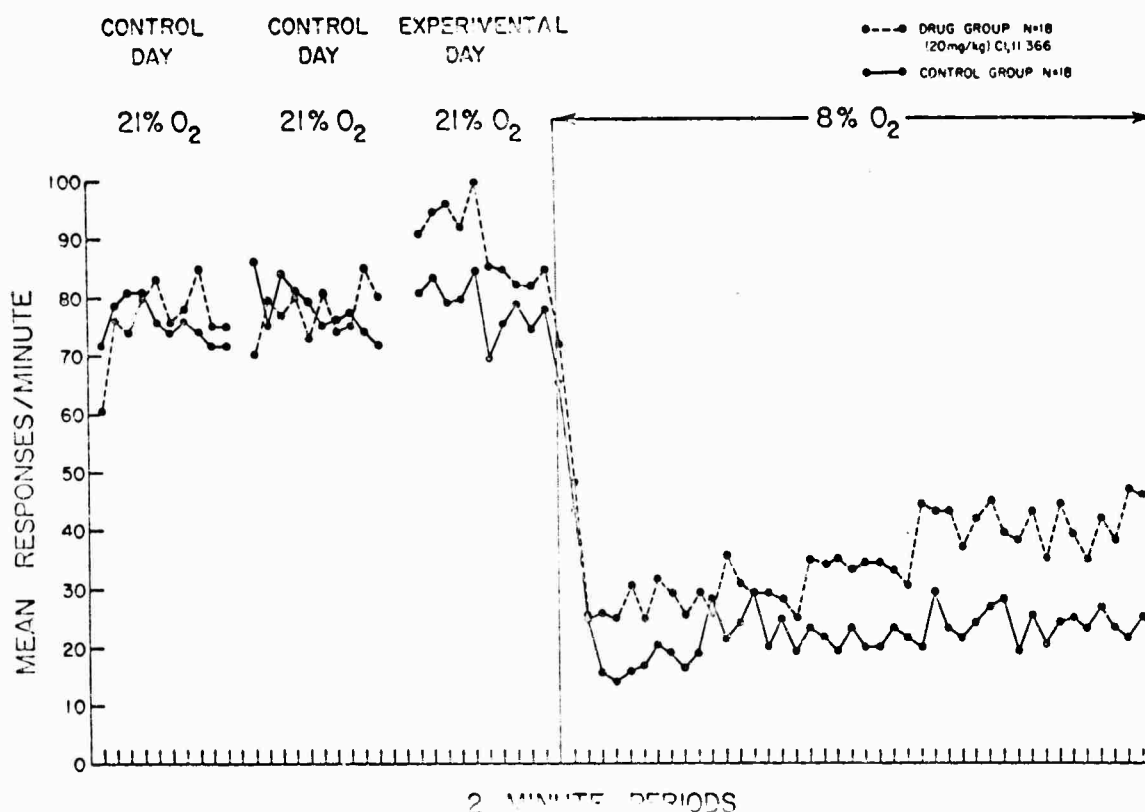
Carbon dioxide has been shown to increase systemic blood pressure and to cause dilation of cerebral arterioles which in turn produces recruitment of additional capillaries. In addition, carbon dioxide causes hyperventilation, a rise in arterial  $pO_2$  and a favorable shift in the oxygen-hemoglobin dissociation curve. All of these factors lead to a higher tissue  $pO_2$  with the effect being most notable on neurons which were on the periphery of the "oxygen cylinders" surrounding the capillaries. These effects prevent neurons which might under normal circumstances drop out of the effective neuronal pool from doing so and thus act in a manner similar to increased stimulating current or increased inspired oxygen.

Stimulating current, oxygen concentration and carbon dioxide concentration all act upon the effective size of the neuronal pool and upon its ability to mediate behavior. This and the preceding report (1967) demonstrate that the rate of self-stimulation can serve not only

as a measure of the percentage activation of the neuronal pool by electrical currents, but as a sensitive measure of the neural response to alterations in oxygen and carbon dioxide concentration. These effects can be observed most effectively when the measurements are made on the steep portion of the sigmoid function relating stimulus intensity and performance.

#### B. The Effects of Carbonic Anhydrase Inhibition on Performance and Neural Reactivity in Low Oxygen Environment

The effects of carbonic anhydrase inhibition on performance in low oxygen environments was investigated using 36 rats trained to self-stimulate. Procedures were similar to those described in the appended manuscripts. Animals were trained to a stable rate of self-stimulation. Eighteen experimental animals were given one dose of 20 mg/Kg Cl<sub>1</sub>,11366 one hour prior to testing. They were then placed in a test chamber in which the oxygen concentration was reduced to eight percent for a two-hour period and self-stimulation rates were monitored. Eighteen control animals were tested in an identical fashion without drug treatment. The results are illustrated in the following figure.



It is clear that the drug group shows a slightly less severe initial drop than the non-drug controls and that a continual improvement in performance during low oxygen exposure results from the drug effects. This study lends additional support to our past findings indicating improved performance in low oxygen environments following carbonic anhydrase inhibition. There is also a slight increase in self-stimulation rates in 21 percent oxygen following carbonic anhydrase inhibition. This indicates a direct effect on neural reactivity in normoxic conditions.

## II. THE EFFECTS OF CARBON DIOXIDE AND/OR CARBONIC ANHYDRASE INHIBITION ON BLOOD GASES AND ACID-BASE BALANCE IN LOW OXYGEN ENVIRONMENTS

The effects of carbon dioxide upon blood gas and acid-base balance in unanesthetized rats were ascertained using the technique described by Mani and Weinstein in determining the effects of carbonic anhydrase inhibition. The results of a study on five catheterized rats are listed in Table I.

In terms of blood gases, one percent carbon dioxide increases arterial oxygen pressure from 25 to 28 mm. of mercury and the pressure of carbon dioxide from 23 to 27 mm. of mercury in eight percent inspired oxygen; whereas a two percent increase in inspired oxygen increases the arterial oxygen pressure from 25 to 30 mm. of Hg, and the pressure of carbon dioxide is increased from 23 to 25 mm. of mercury. This is illustrated in Figures 6-7 (pp 13-14). The behavior in the two situations is identical, and we would conclude that arterial oxygen pressure and the pressure of carbon dioxide might both be determining performance.

Table I  
Blood Gas and Acid-Base Changes with Hypoxia and Hypercapnia in  
Unanesthetized Rats

	pH	PO <sub>2</sub>	PCO <sub>2</sub>
Air	7.413	72.4	34.8
14% O <sub>2</sub>	7.445	49.0	31.7
14% O <sub>2</sub> + 1% CO <sub>2</sub>	7.448	56.0	37.0
14% O <sub>2</sub> + 2% CO <sub>2</sub>	7.455	61.4	34.1
14% O <sub>2</sub> + 3% CO <sub>2</sub>	7.426	69.7	38.2
Air	7.426	73.3	35.1
12% O <sub>2</sub>	7.509	38.7	28.1
12% O <sub>2</sub> + 1% CO <sub>2</sub>	7.479	44.9	32.3
12% O <sub>2</sub> + 2% CO <sub>2</sub>	7.485	50.0	35.1
12% O <sub>2</sub> + 3% CO <sub>2</sub>	7.452	55.4	34.4
Air	7.459	75.0	37.2
10% O <sub>2</sub>	7.588	29.7	24.8
10% O <sub>2</sub> + 1% CO <sub>2</sub>	7.546	35.5	25.2
10% O <sub>2</sub> + 2% CO <sub>2</sub>	7.492	41.1	33.2
10% O <sub>2</sub> + 3% CO <sub>2</sub>	7.486	46.4	30.0
Air	7.457	76.3	40.4
8% O <sub>2</sub>	7.590	25.1	23.2
8% O <sub>2</sub> + 1% CO <sub>2</sub>	7.489	28.4	27.0
8% O <sub>2</sub> + 2% CO <sub>2</sub>	7.495	31.9	32.1
8% O <sub>2</sub> + 3% CO <sub>2</sub>	7.462	38.7	35.9
Air	7.452	71.5	39.3
21% O <sub>2</sub> + 1% CO <sub>2</sub>	7.428	80.2	41.1
21% O <sub>2</sub> + 2% CO <sub>2</sub>	7.439	79.4	42.9
21% O <sub>2</sub> + 3% CO <sub>2</sub>	7.407	85.0	40.5

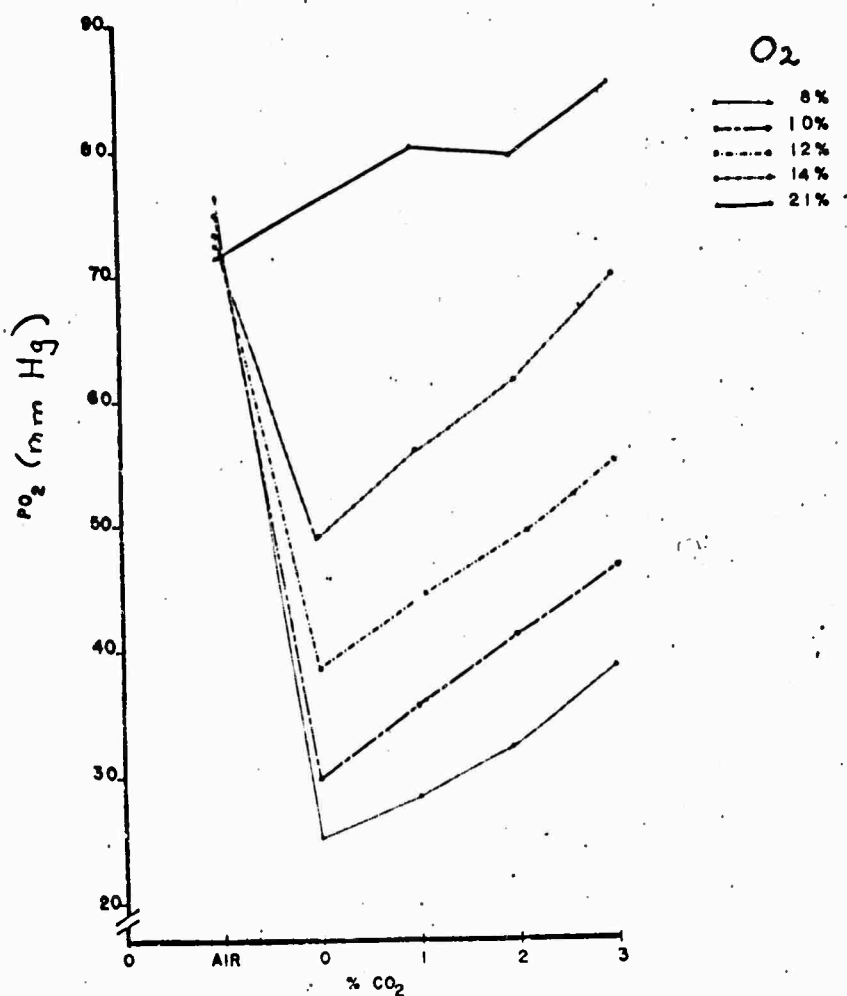


Fig. 6

Changes in oxygen pressure with increased carbon dioxide content

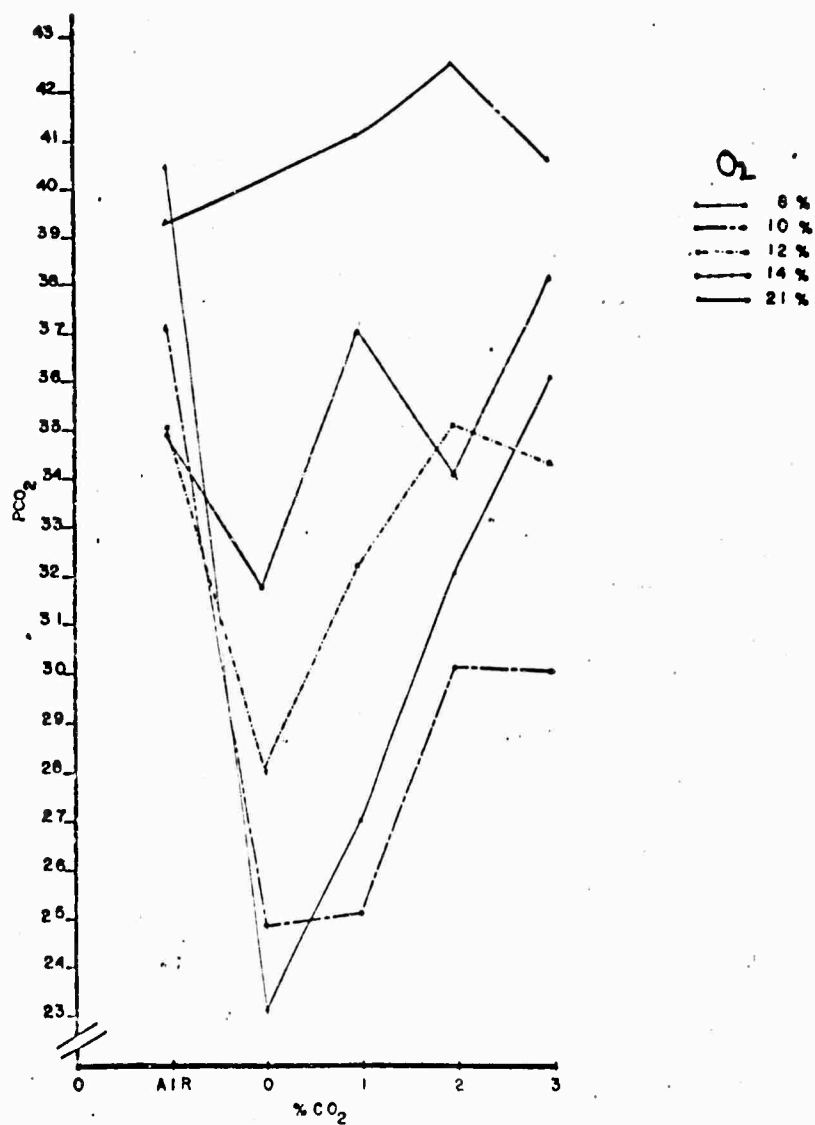


Fig. 7

Changes in carbon dioxide pressure with increased CO<sub>2</sub> content

If we now look at arterial pH, we can see that one percent carbon dioxide drops pH in eight percent oxygen from 7.59 to 7.49. There is, however, no significant drop in pH when the inspired oxygen is increased to ten. This can be seen in Figure 8. We must now establish whether pH determines performance in low oxygen environments. In order to do this, we have administered a carbonic anhydrase inhibitor Cl 11,366 in a dose of 20 mg/Kg, one hour before a two-hour low oxygen exposure. Carbonic anhydrase inhibition increased self-stimulation rates in eight percent oxygen from 22 responses per minute to 40 responses per minute which was significant at the .025 level. In terms of blood gas and acid-base balance, the drug administered in this way increased the oxygen pressure only 1.7 mm of Hg (which was not significant) and carbon dioxide increased only one millimeter of mercury (not significant); the pH decreased from 7.63 to 7.52. This pH decrease with minimal oxygen and carbon dioxide increase produces a behavioral improvement equivalent to the addition of 1.0 percent carbon dioxide or two percent oxygen. It thus seems likely that all three factors -- pH,  $pCO_2$ , and  $pO_2$  -- are capable of altering neural function and performance in low oxygen environments.

These data illustrate that physiological and behavioral measures, when in a quantitative form, can be highly predictive and of significant theoretical use.

### III. THE EFFECTS OF "MOTIVATION" ON PERFORMANCE IN HYPOXIC ENVIRONMENTS

It is well known that the level of activity of the CNS is a function of the level of tissue oxygen. Numerous studies in the past have shown that hypoxia has deleterious effects on performance. More recently, Creutzfeldt has shown that in anoxia the number of active cortical cells declines sharply; and Woolley and Timiras have found that synaptic delay

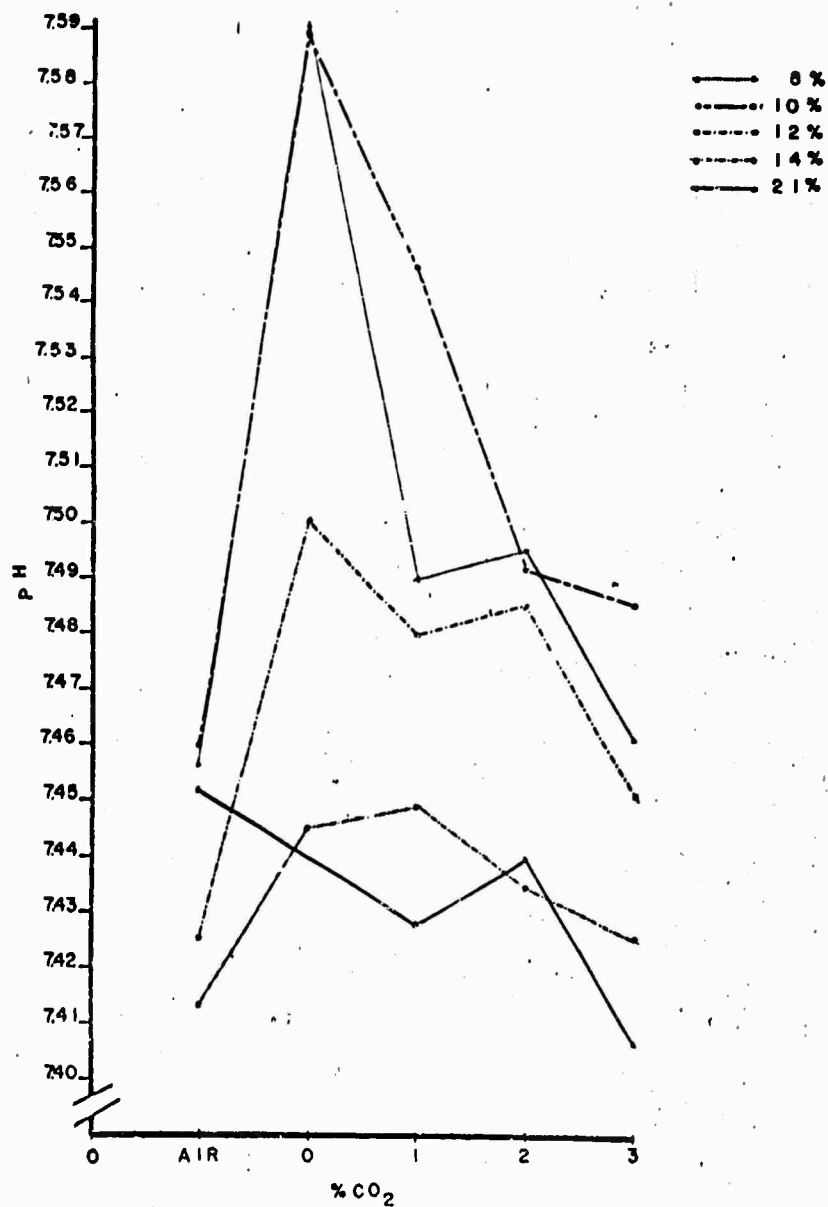


Fig. 8

Changes in pH with increased carbon dioxide content

and conduction time increase at simulated high altitudes. These studies, however, do not reveal how changes in the level of induced activity in the CNS interact with the effects of hypoxia. Our investigations were undertaken in order to investigate systematically the effects of hypoxia on a skilled motor task (lever pressing or brain stimulation) and, further, to determine the interaction between hypoxia and changes in CNS activity due to changes in the intensity of the brain stimulating current.

Our results indicate that motivation (as measured by the amount of emitted behavior in air) is of major importance in the determination of the effects of hypoxia on performance. Figure 9 A illustrates this clearly. At the high motivational level (Fig. 9 A), 14 percent and 12 percent oxygen produces a slight impairment in performance, but performance dropped sharply in 10 percent and 8 percent oxygen. At the lower motivational level, however, performance decreased markedly even at 14 percent oxygen, and further decreases in the oxygen concentration impaired performance only slightly more. A series of nonparametric sign tests showed that performance at all levels of hypoxia differed significantly ( $p < .008$ ) from the air control rates at both the high and low motivational levels. A comparison of the high and low motivational states showed that performance in 21 percent, 14 percent, and 12 percent oxygen was significantly better in the high motivational state than in the low motivational state ( $p < .008$ ). There were no significant differences in performance between the two motivational states in 10 percent and 8 percent oxygen. These results suggest that the two curves have different slopes and that there is an interaction between motivational level and performance in hypoxia. (See Appendix B)

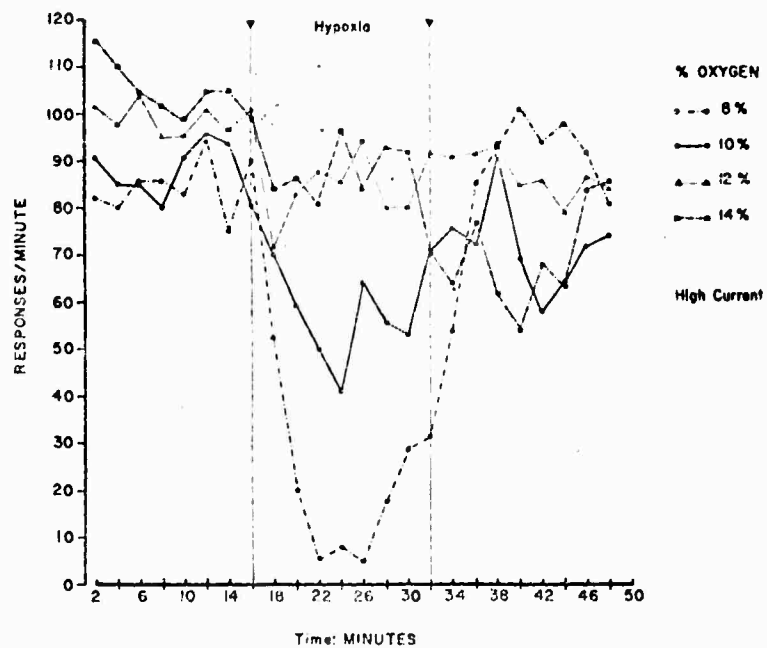


FIG. 9A

The effect of inspired oxygen on the rate of self-stimulation as a function of rewarding current intensity. Fig. 9A shows lever pressing rates for successive two-minute period for the "high" motivation group in air (0-16 min.), in hypoxia, (16-32 min), and in air again (32-48 minutes).

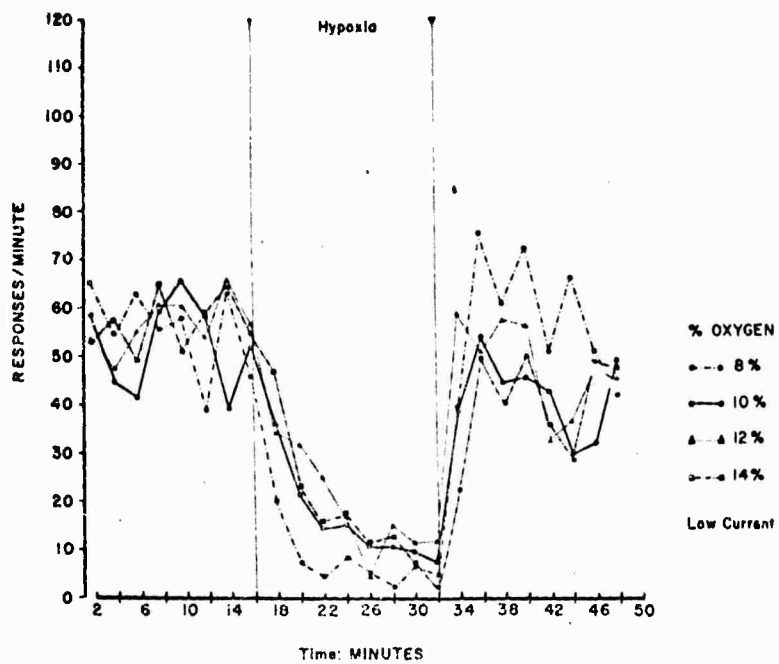


FIG. 3 B

The effect of inspired oxygen on the rate of self-stimulation as a function of rewarding current intensity. Fig. 3 B illustrates the performance of the same animals in the "low" motivation state.

The results of these experiments clearly establish that the degree of impairment of performance by hypoxia is a function of the response rate or "motivational level" of the animal.

Several hypotheses can be advanced to account for these results. First, the data of Olds should be considered, describing the relationship between rate of responding and current intensity. With electrodes in the posterior lateral hypothalamus, Olds found that this relationship could be described by a sigmoid function. When animals were pressing at very high rates, decreases in current intensity produced only small decrements in response rates. At the lower end of the function, decreases in current intensity produced precipitous declines in response rates and additional decreases in current produced no further changes. The behavior of the animals in the present experiment with electrodes in the posterior lateral hypothalamus, seems to follow the model outlined by Olds, if one assumes that the effect of hypoxia is similar to a decrease in the intensity of the stimulating current. This assumption is supported by the data of Creutzfeldt on the effects of anoxia on cortical cell function. Creutzfeldt found that the activity of the cells underwent three distinct phases as the oxygen level dropped. Phase 1 was characterized by an increase in the frequency of discharges and also by an increase in the regularity of the discharges. In Phase 2, only about fifty percent of the neurons fired, and the EEG was characterized by delta waves. In Phase 3, all electrical activity stopped. Olds suggests that increasing stimulus strength increases the number of cells activated and that this increase in number of active cells is the basis for higher response rates (high motivational level). Creutzfeldt's data indicate that hypoxia, in Phase 2, produces decreases in the number of active cells, thus providing a reasonable basis for predicting lower response rates at a fixed current level during hypoxia.

There are several other factors which should be taken into consideration. We have previously demonstrated that alteration of cerebral blood flow can markedly affect the rate of self-stimulation during hypoxia. The addition of three percent carbon dioxide to the inspired air can move performance in eight percent oxygen back to control levels. The administration of 2-Benzenesulfonamido-134 Thiadiazole-5-Sulfonamide (a potent carbonic anhydrase inhibitor) produces an increased rate of responding equivalent to the addition of 1.5 percent carbon dioxide (unpublished observation). Excitation of neural tissue has been shown to increase local cerebral blood flow; this effect may be graded to produce higher blood flows with higher levels of excitation. Increases in blood pressure which might also increase cerebral blood flow have been demonstrated to occur with stimulation of the hypothalamus. In addition, the changes in glucose levels which have been observed might also interact with performance during hypoxia. These factors probably take part in determining the absolute level of performance at both high and low motivational levels; the major difference between the effects of hypoxia on the two levels of performance can probably be accounted for by the stimulus strength versus response rate function.

#### IV. THE EFFECTS OF PAST EXPERIENCE IN LOW OXYGEN UPON PERFORMANCE DURING SUBSEQUENT EXPOSURES

##### A. Introduction.

It is well established that continued exposure to low oxygen environments ("high altitude") results in a set of physiological adaptations which are lumped under the designation of "acclimatization." These changes are distinguished from the immediate physiological changes to a decreased oxygen concentration by their gradual onset and development. It has generally been assumed that continuous and prolonged exposure

to diminished oxygen is required for acclimatization to occur. We will now describe a set of events which occur as a result of successive, brief (16 minute) exposures to low oxygen. The relationship between these events and classical measures of acclimatization indicate that the process is independent of changes in blood gases, acid-base balance and hematocrit. The changes that occur are characterized by an improvement in performance on successive daily tests in low oxygen. Passive brief exposure to low oxygen does not produce improved performance and there appears to be a significant interaction between what the animal has done previously in the environment and what he will do in the future.

#### B. Materials and Methods

The subjects were four-month old, naive male, hooded rats, maintained on an ad lib. diet. Thirty-two animals had monopolar stainless steel electrodes implanted in the medial forebrain bundle. Using surface coordinates on the skull, the electrodes were implanted 4.5 mm posterior to the bregma, 1.5 mm lateral to the midline and 8.5 mm below the surface of the skull. One week after surgery, the animals were trained to self-stimulate in a modified Skinner box. This box has been described in detail elsewhere. Briefly, a small lever at one end of the box activated the brain stimulation current; a constant current 110 v, 60 cps device delivered a 250 ms pulse on each depression of the lever. The effluent from a gas mixing device was connected to the Skinner box, and the rate of flow of the various gas mixtures was held constant at 24 l/m. The intensity of the brain stimulating current was adjusted to produce lever pressing rates of approximately one hundred presses per minute in 21 percent oxygen. When the animals had stabilized at these rates, the experimental sessions were started.

There are several other factors which should be taken into consideration. We have previously demonstrated that alteration of cerebral blood flow can markedly affect the rate of self-stimulation during hypoxia. The addition of three percent carbon dioxide to the inspired air can move performance in eight percent oxygen back to control levels. The administration of 2-Benzenesulfonamido-134 Thiadiazole-5-Sulfonamide (a potent carbonic anhydrase inhibitor) produces an increased rate of responding equivalent to the addition of 1.5 percent carbon dioxide (unpublished observation). Excitation of neural tissue has been shown to increase local cerebral blood flow; this effect may be graded to produce higher blood flows with higher levels of excitation. Increases in blood pressure which might also increase cerebral blood flow have been demonstrated to occur with stimulation of the hypothalamus. In addition, the changes in glucose levels which have been observed might also interact with performance during hypoxia. These factors probably take part in determining the absolute level of performance at both high and low motivational levels; the major difference between the effects of hypoxia on the two levels of performance can probably be accounted for by the stimulus strength versus response rate function.

#### IV. THE EFFECTS OF PAST EXPERIENCE IN LOW OXYGEN UPON PERFORMANCE DURING SUBSEQUENT EXPOSURES

##### A. Introduction.

It is well established that continued exposure to low oxygen environments ("high altitude") results in a set of physiological adaptations which are lumped under the designation of "acclimatization." These changes are distinguished from the immediate physiological changes to a decreased oxygen concentration by their gradual onset and development. It has generally been assumed that continuous and prolonged exposure

to diminished oxygen is required for acclimatization to occur. We will now describe a set of events which occur as a result of successive, brief (16 minute) exposures to low oxygen. The relationship between these events and classical measures of acclimatization indicate that the process is independent of changes in blood gases, acid-base balance and hematocrit. The changes that occur are characterized by an improvement in performance on successive daily tests in low oxygen. Passive brief exposure to low oxygen does not produce improved performance and there appears to be a significant interaction between what the animal has done previously in the environment and what he will do in the future.

#### B. Materials and Methods

The subjects were four-month old, naive male, hooded rats, maintained on an ad lib. diet. Thirty-two animals had monopolar stainless steel electrodes implanted in the medial forebrain bundle. Using surface coordinates on the skull, the electrodes were implanted 4.5 mm posterior to the bregma, 1.5 mm lateral to the midline and 8.5 mm below the surface of the skull. One week after surgery, the animals were trained to self-stimulate in a modified Skinner box. This box has been described in detail elsewhere. Briefly, a small lever at one end of the box activated the brain stimulation current; a constant current 110 v, 60 cps device delivered a 250 ms pulse on each depression of the lever. The effluent from a gas mixing device was connected to the Skinner box, and the rate of flow of the various gas mixtures was held constant at 24 l/m. The intensity of the brain stimulating current was adjusted to produce lever pressing rates of approximately one hundred presses per minute in 21 percent oxygen. When the animals had stabilized at these rates, the experimental sessions were started.

### Experiment I

In order to investigate the effects of repeated brief exposures to eight percent oxygen on self-stimulation rates, twenty-one rats were randomly assigned to three experimental groups of seven animals each.

Animals in Group A were allowed to self-stimulate for 48 minute sessions every day. During the first 16 minutes, the animals were in a normoxic environment (21 % O<sub>2</sub>). During the next 16 minute period, the oxygen concentration was lowered to eight percent with a change time of two minutes. During the last 16 minute period, the animals were again breathing 21 percent oxygen. This procedure was repeated daily for seven days. In order to determine whether the change in performance during hypoxia was controlled by a "time" or a "frequency" factor, another group of seven animals were exposed to hypoxia (8% O<sub>2</sub>) only every third day. The experimental sessions lasted 48 minutes every day, and the animals were exposed to hypoxia, during the middle 16-minute period of the session every third day. This procedure was repeated until the animals had been exposed to hypoxia seven times.

Finally, in order to determine whether passive exposure to hypoxia could alter subsequent lever pressing performance in hypoxia, seven animals in "Group C," were placed in the Skinner box for three days, 16 minutes per day, in eight percent oxygen, with an obstruction placed between the animals and the lever. Three hours later, the animals were allowed to self-stimulate in air for 48 minutes. These animals were subsequently trained under the same conditions as Group A, for seven days, i.e. a 16-minute exposure to eight percent oxygen every day preceded and followed by 21 percent oxygen. The number of lever presses throughout the experiment were recorded every two minutes by standard programming equipment.

### Experiment II

In this experiment, eleven rats with hypothalamic electrodes were used to assess the effects of repeated brief exposures to 11 percent oxygen. The procedure was essentially the same as described above for Group A. During daily 48 minute lever pressing sessions, the animals were run in 21 percent oxygen during 16 minutes, in 11 percent oxygen during the next 16 minutes and in 21 percent oxygen during the last 16 minutes. This procedure was repeated for six days. Because of the minimal effects of this mild hypoxia upon performance, only one group of animals was run.

### Experiment III

Since a change in performance during repeated exposures to hypoxic environments could be attributed to either some behavioral or physiological adaptation mechanism, it was important to investigate the effect of these exposures on possible changes in the concentration of blood gases. Consequently, nine animals had catheters implanted in the abdominal aorta using a procedure described previously. The catheters were anchored on the skull with dental cement, and the animals were completely free to move in their cages while blood samples were being taken. When the animals had recovered from surgery, they were placed in the Skinner box used in experiments I and II and exposed to eight percent oxygen for 16 minutes every day for seven days. At the end of the 16-minute period, blood samples were taken from each animal (0.1 cc) and the pH,  $pO_2$ , and  $pCO_2$  were determined using an IL microsample analyzer. A second blood sample of 0.1 cc was taken in order to determine the hematocrit and the oxygen saturation using a Radiometer analyzer. Following this procedure, the animals were returned to 21 percent oxygen.

### Results

The results of Experiment I are illustrated in Figure 10. This Figure shows the performance of Groups A, B and C in 21 percent oxygen as well as in eight percent oxygen for seven days. As can be seen, the performance of all three groups in 21 percent oxygen fluctuated around 100 lever presses per minute. Performance in eight percent oxygen for Group A (run every 24 hours) and B (run every 72 hours) dropped to 50 responses per minute on Day 1 and then increased with every hypoxic exposure. The performance of Group C (passive pre-exposure to eight percent oxygen) was below the level of Groups A and B and improvement was considerably delayed. By Day 8, however, all groups exposed to eight percent oxygen seemed to be performing at the same level.

An analysis of variance of the mean number of responses per minute made by Groups A, B and C over seven days in 21 percent oxygen showed only a day by day treatment interaction ( $p < .05$ ). Examination of Fig. 1 shows that while the performance of Group B deteriorated over the seven days, the performance of Group C improved to a higher level than the other groups.

Analysis of the rate of lever pressing for the three groups in eight percent oxygen revealed a significant day effect ( $p < .01$ ) and a significant day by treatment interaction ( $p < .01$ ). It can be seen from Fig. 1 that although all groups improved markedly during the seven days, Group C improved very little until days six and seven when the performance of the animals suddenly rose to the level of the other groups. This would seem to indicate that passive exposure to hypoxia retarded the improvement in lever pressing rates considerably.

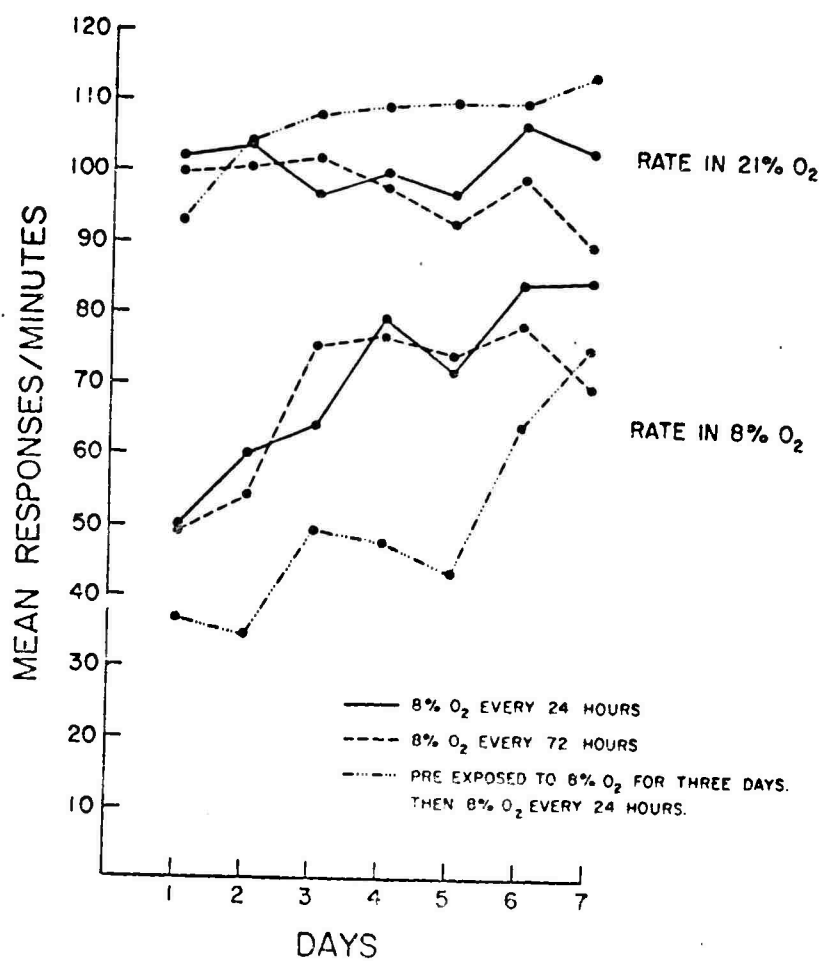


Fig. 10

The daily difference between the animals' performance in 21 percent oxygen and in eight percent oxygen was evaluated by separate analyses of variance. These analyses indicated that on Days 1 and 2 the animals were performing significantly better ( $p < .001$ ) in 21 percent oxygen than in eight percent oxygen. On Days 3, 4, and 5, there was a significant difference between rates in 21 percent oxygen and eight percent oxygen as well as a significant interaction between experimental conditions and oxygen level ( $p < .05$ ). It can be seen from Fig. 10 that Group C on Days 3, 4, and 5 was performing not only worse than the other groups in eight percent oxygen, but seemed to have reached a plateau. On Days 6, and 7, this interaction effect was no longer significant and only the difference between the performance in 21 percent oxygen and eight percent oxygen remained significant ( $p < .001$ ). These analyses show that despite the obvious improvement in performance in eight percent oxygen during the seven days, the animals were never able to overcome behaviorally the oxygen deficit.

#### Experiment II

#### Performance

deteriorated only slightly in eleven percent oxygen on Day 1, and the animals showed rapid improvement on subsequent days. The analysis of variance comparing performance in 21 percent oxygen with performance in ten percent oxygen over the six days showed no significant effects. The daily difference between performance in 21 percent oxygen and 11 percent oxygen was analyzed by Wilcoxon Rank sum tests. These analyses revealed that on Days 1 and 2, performance in 21 percent oxygen was significantly higher ( $p < .05$  on Day 1, and  $p < .025$  on Day 2) than in eleven percent oxygen. After Day 2, however, there were no significant differences between performance levels in 21 percent oxygen or eleven percent oxygen.

### Experiment III

The results of the analyses of blood gases, oxygen saturation, and hematocrit are illustrated in Figs. 11-15. As can be seen, none of these variables showed any significant changes during the seven days of exposure. There were no statistical differences between the results obtained from Days 1 to 7. This would indicate that the behavioral changes observed in Experiment I were not accompanied by physiological changes in the blood that one would normally expect to accompany physiological adaptations to low oxygen environments.

### Discussion

These results indicate that successive daily exposure to low oxygen results in significant increase in hypothalamic self-stimulation rates. This improvement occurs independently of the acid-base and respiratory balance maintained by the animals. There is, however, a significant interaction between what the subjects have done in hypoxia in the past and what they will do in the future. The results of Experiment I clearly illustrate that passive pre-exposure results in a significantly retarded rate of behavioral adaptation to low oxygen exposure. This suggests that the factors responsible for improved performance are not simply triggered by hypoxia, but are probably the results of specific adaptations by the neuronal pool which mediates the self-stimulation behavior. In earlier studies we found that the effects of hypoxia interacted with motivational level in a rather striking fashion. Animals self-stimulating at low rates with low current levels show marked suppression in mild hypoxia (14 %  $O_2$ ). This suppression can be reversed by increasing the stimulating current. If the subject is in a severely hypoxic state (8 %  $O_2$ ), the deterioration cannot be reversed by an increase in current. Addition of carbon dioxide or administration of a carbonic anhydrase inhibitor will alleviate this

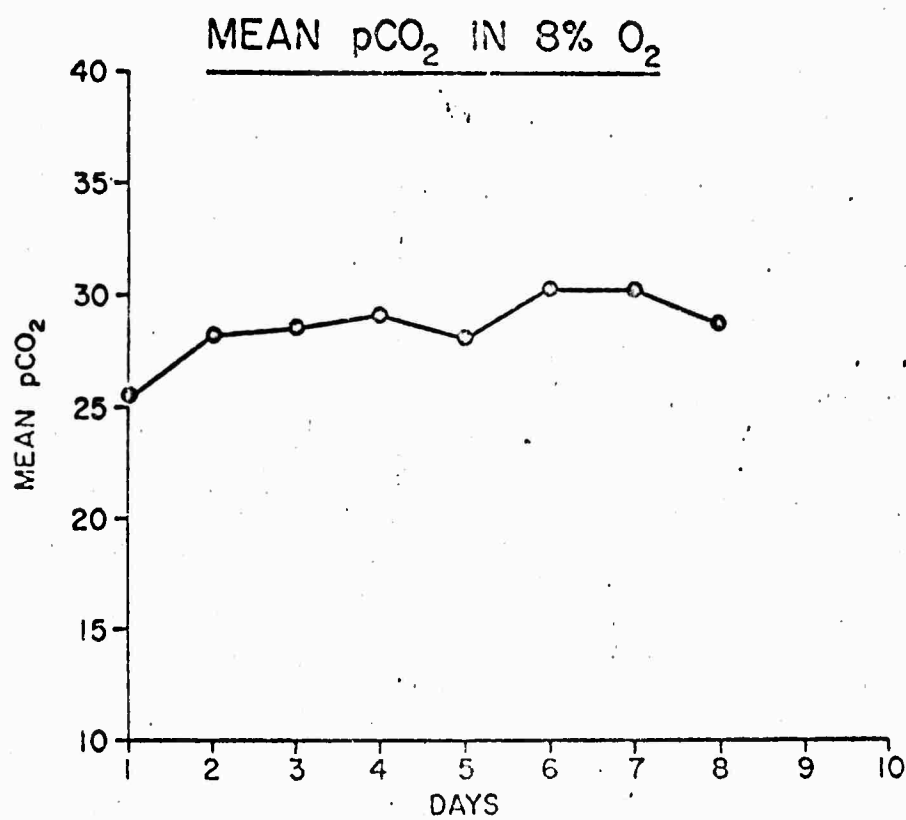


Fig. 11

Changes in Mean  $p\text{CO}_2$  in 8 percent  $\text{O}_2$  over an eight-day period.

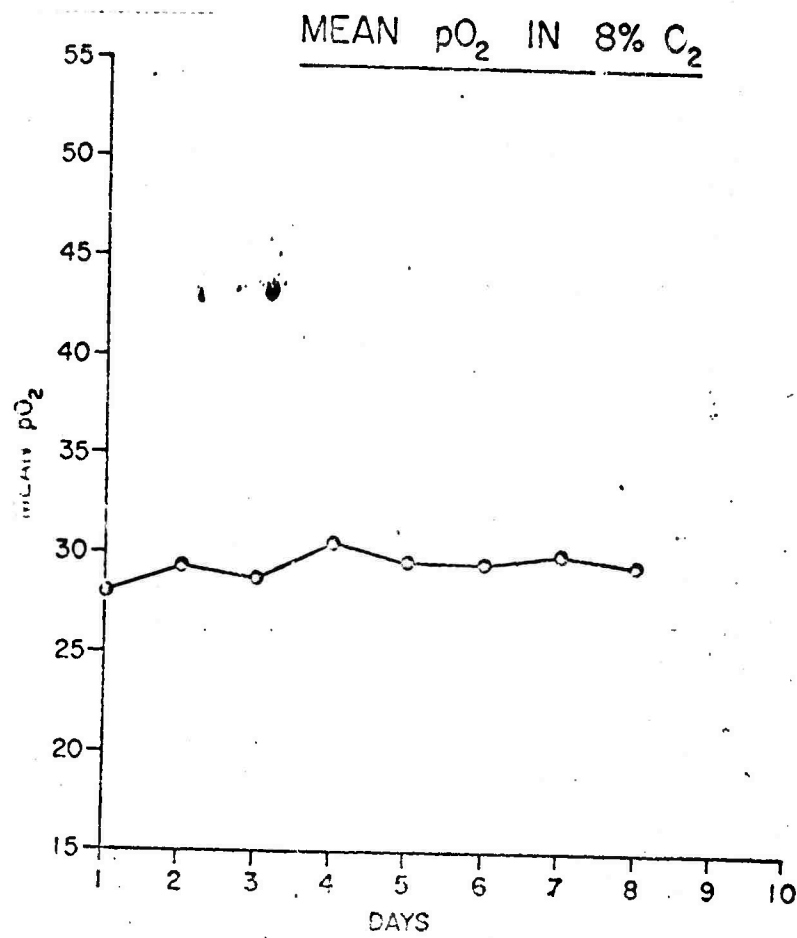


Fig. 12

Changes in Mean  $pO_2$  in 8 percent  $O_2$  over an eight-day period

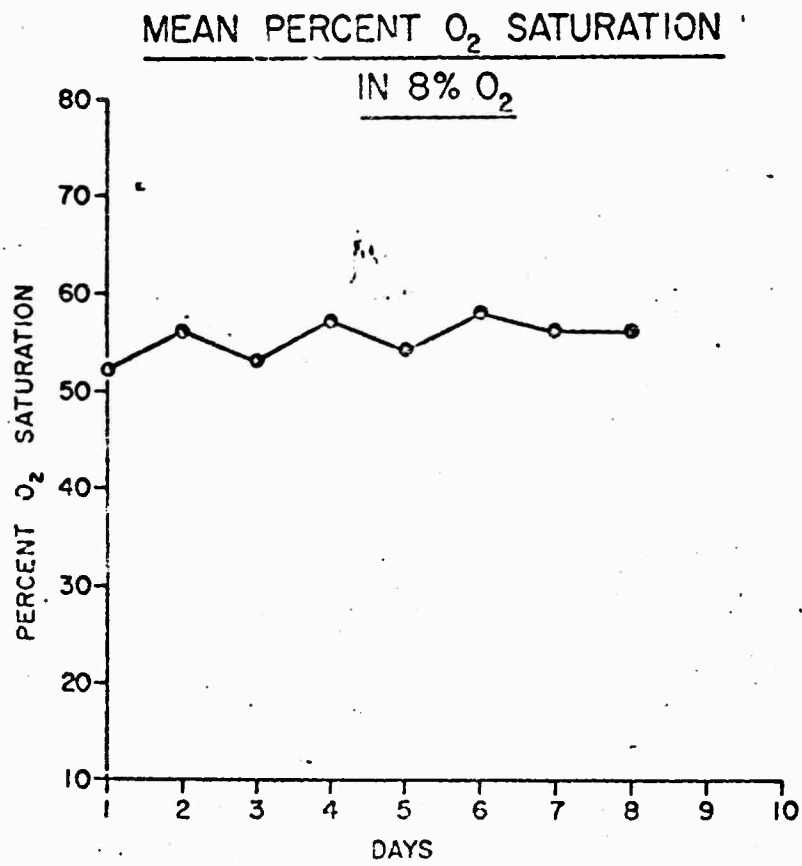


Fig. 13

Changes in Percent O<sub>2</sub> Saturation in 8 percent O<sub>2</sub> over an eight-day period

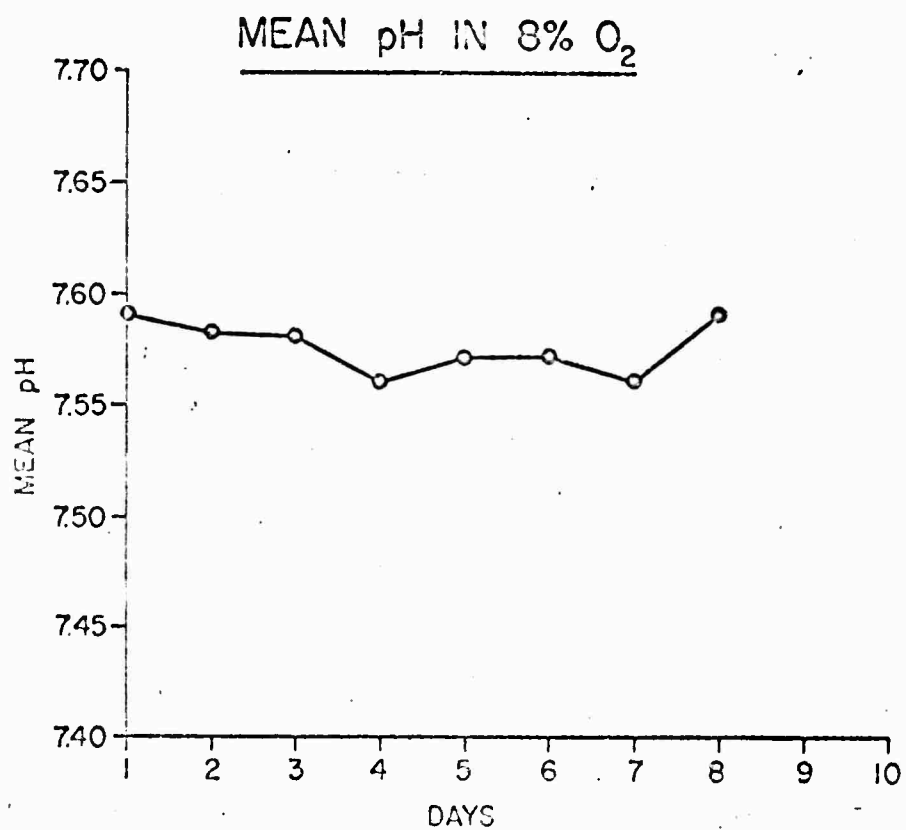


Fig. 14

Changes in Mean pH in 8 percent O<sub>2</sub> over an eight-day period

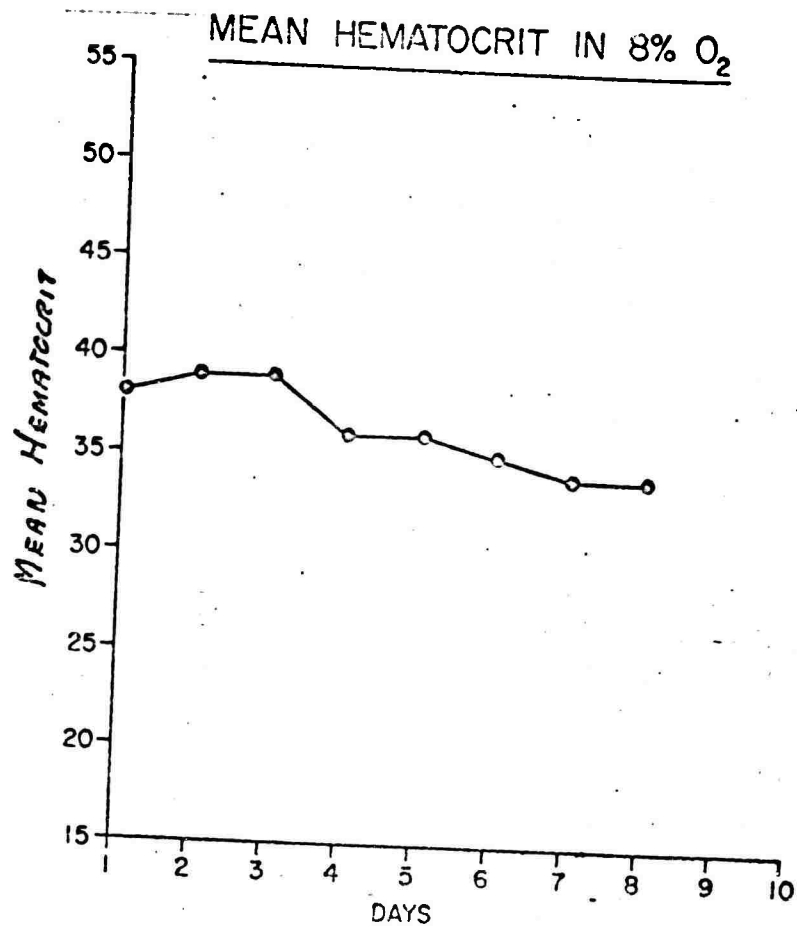


Fig. 15

Changes in Mean Hematocrit in 8 percent O<sub>2</sub> over an eight-day period

deterioration. This indicates that an improvement in the state of the already activated neuronal pool is required for improved performance rather than an enlargement of the activated area by increased current levels. As of this time, we have no basis for speculating as to the nature of these changes.

#### V. THE EFFECTS OF SCHEDULES OF REINFORCEMENT UPON PERFORMANCE IN LOW OXYGEN

We have now run a series of six pigeons on two schedules of reinforcement, fixed interval (FI) and fixed ratio (FR). The FI schedule is characterized by a slow rate of responding during the early part of the interval with a gradual increase in response rate toward the end of the interval. It is inferred that there is an active inhibition of responding during the early part of the FI. The fixed ratio (FR) schedule is characterized by a continuous, high rate of response with inhibition occurring only at the time of reinforcement. These two schedules of reinforcement are affected in a very dissimilar way by hypoxia. Pigeons on an FI schedule show an increased response rate to hypoxia (as low as 8 %  $O_2$ ). The same pigeon on FR shows a marked decrease in response rate at the same oxygen level. It thus appears that the directional change in rate of responding is not a good indication of the effects of hypoxia. The increased rate of responding produced by hypoxia is probably due to the impairment of function of inhibitory neurons during the initial phase of the FI. This work is just beginning and more detailed information will be available at a later date.